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INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 5 : A61K 37/02, 47/26, 47/42 A61K 47/18, 9/14		A1	(11) International Publication Number: WO 93/23065 (43) International Publication Date: 25 November 1993 (25.11.93)
(21) International Application Number: PCT/EP93/01120			(81) Designated States: AU, BR, CA, FI, JP, KR, NO, RU, US, European patent (AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE).
(22) International Filing Date: 7 May 1993 (07.05.93)			
(30) Priority data: RM92A000349 11 May 1992 (11.05.92)		IT	Published <i>With international search report. Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.</i>
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(54) Title: PHARMACEUTICAL COMPOSITIONS CONTAINING IL-6

(57) Abstract

Pharmaceutical compositions based on Interleukin-6 (IL-6) stabilized with non reducing sugars, such as sucrose and trehalose. The compositions may also contain an amino acid or human albumin as an excipient. The formulation is particularly suitable for the stabilization of recombinant IL-6 freeze-dried powder.

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PHARMACEUTICAL COMPOSITIONS CONTAINING IL-6

The present invention contemplates pharmaceutical compositions containing Interleukin-6 (IL-6), and particularly contemplates compositions based on IL-6 stabilized with nonreducing sugars.

Interleukin-6 is a protein belonging to the group of cytokines, which proved to play a key role in the organism immune response and haematopoiesis stimulation (International Symposium on IL-6: Physiopathology and Clinical Potentials, Montreux, October 21-23, 1991).

The prospective therapeutic applications of IL-6 are tumoral growth inhibition, treatment of thrombocytopenia caused by chemotherapy, radiotherapy, and even accidental exposure to radiations. It may also be used as a vaccine adjuvant.

According to the present invention, IL-6 may be either natural or synthetic, i.e. produced on the basis of recombinant DNA technology, the latter being preferred.

The protein of this invention is glycosylated human IL-6, prepared on the basis of the recombinant DNA technology by expression in CHO (Chinese Hamster Ovary) cells, transformed with the corresponding DNA, according to the disclosures of European Patent Application EP 0220574.

As known, purified proteins show a great tendency to become denatured, even by normal atmospheric agents. This characteristic is even more evident in proteins produced on the basis of recombinant DNA technology. To prevent any contamination of non-

human origin, they must be purified to a high degree, which makes their stability lower than that of corresponding purified natural proteins.

IL-6 formulations for injection are obtained on the basis of a
5 process inclusive of freeze-drying for dry powder production.

As described by M.J. Pikal in Biopharm., October 25-30, 1990, the protein pharmacological activity is reduced by phenomena taking place during freeze-drying.

For example, proteic aggregates, which are generally regarded as
10 directly responsible for the onset of allergic manifestations, frequently form during the process. Furthermore, should the protein be not damaged by the various process stresses, a partial denaturation of same during storage operations would be extremely probable.

15 It is just because of the very easy denaturation of highly purified proteins that it is highly desirable to produce stable formulations with an as long life cycle as possible, even when stored at ambient temperature.

The expression "formulation stability" is used to mean that the
20 protein maintains its activity both during the pharmaceutical preparation and storage.

The formulations containing highly purified proteins may be stabilized by addition of one or more excipients, preventing or delaying the active ingredient degradation.

25 Excipients of different chemical nature were used in various proteins formulations.

High molecular weight stabilizers of biological origin, such as sea colloids, dextran, and phospholipids, are known.

Equally effective stabilizers often proved to be the formulations containing proteins, e.g. albumin, amino acids, e.g. arginine or 5 glycine, and sugars, e.g. monosaccharides or oligosaccharides.

Another cytokin, i.e. Interleukin-2 (IL-2), and particularly its recombinant form, was formulated with various stabilizers, preferably albumin and amino acids.

International patent application WO 90/00397 discloses IL-2 10 stabilization with arginine or carnitine or a mixture thereof, with betaine, pyridoxine, polyvinylpyrrolidone, carboxylic acids salts, and by the addition, if any, of other excipients, such as sugars and citrate buffer.

European patent application EP 158487 discloses IL-2 formulations 15 with human albumin and a reducing compound, such as glutathione, N-acetylcysteine or ascorbic acid.

Pikal in Biopharm., October 25-30, 1990, also suggests that excipients capable of bringing about amorphous and/or vitreous structures can cause protein stabilization on drying.

20 The amorphous structure seems to secure a considerable restriction of protein molecular mobility, with consequent decrease in chemical reactivity, as well as a long lasting protection: in fact, it is supposed to form a sort of casing where the protein is housed and, therefore, protected also after the process cycle.

25 However, Pikal states that an amorphous excipient is not sufficient

for stability increase. Actually, the protein may be denatured just by interacting with the amorphous excipient.

The conclusion is that a general criterion for proteins formulation cannot be put forward: the optimal formulation composition can be
5 determined only through an exacting work of screening of a large number of substances.

The study of a new protein, such as IL-6, required an in-depth investigation of various stabilizing agents, including the substances that give an amorphous structure, such as nonreducing
10 sugars.

It has surprisingly been found that nonreducing sugars, such as for example sucrose and trehalose, increase the stability of IL-6 formulation.

It is the main object of the present invention to provide a
15 pharmaceutical composition containing an intimate mixture of IL-6 and a stabilizing quantity of a nonreducing sugar either alone or in conjunction with other excipients.

It is a further object of the present invention to provide a procedure for the preparation of said pharmaceutical composition,
20 including the components aqueous solution freeze-drying.

It is a further object of the present invention to provide a form of said pharmaceutical composition in which the aforesaid intimate solid mixture is hermetically enclosed in a sterile container suitable for storage before use and for the mixture reconstitution
25 in a solution for injection.

It is a further object of the present invention to provide a

solution of said solid mixture reconstituted in a solution for injection.

With a view to evaluating the excipient effect on the active ingredient stability, several formulations of recombinant IL-6 containing 35 µg/vial were prepared with various excipients, such as mannitol, sucrose, trehalose, lactose mixed with an amino acid, such as arginine or glycine, or with human serum albumin (HSA).

Table 1 shows the composition of the various formulations prepared (A1, A2, A3, A4, etc.), expressed as content (in mg) per vial.

All formulations contain arginine or glycine or human serum albumin (HSA) in addition to other excipients.

Table 1
Formulations of recombinant IL-6 (35 µg)
(content/vial)

COMP. FORM.	HSA mg	Mannitol mg	Saccharose mg	Trehalose mg	Lactose mg	Arginine HCl mg	Glycine mg	Na ₂ HPO ₄ mg	Na ₂ HPO ₄ mg
A1	0.2	25			0.125	0.5		0.313	0.336
A2	0.2	25			0.125		0.313	0.336	
A3	0.2		47.5		0.125		0.313	0.336	
A4		25			0.125		0.313	0.336	
A5			47.5		0.125		0.313	0.336	
A6				47.5	0.125		0.313	0.336	
A7					1.5		0.313	0.336	
A8					1.5		0.313	0.336	
A9					44.5	1.5	0.313	0.336	
A10						1.5	0.313	0.336	
						10.4			

The freeze-dried powder was obtained on the basis of the following process: IL-6 bulk was diluted with the excipient solution in phosphate buffer at pH 7. The solution obtained was filtered, made up to volume, poured into the vials, and freeze-dried.

5 The samples were maintained at 50°C and subjected to immunologic- and bioassays at set time intervals.

The immunologic assay was carried out using QUANTIKINE kit, (R&D SYSTEMS Inc.), cat. No. D6050, following the instructions attached thereto.

10 The bioassay was carried out as described by Normann and Potter in Science, 233, 566-569, 1980. The assay measures IL-6 activity by exploiting IL-6 capability of acting as a growth factor of a particular cell line (plasmacytoma T-1165).

15 Activity is expressed in international units/solution milliliter (IU/ml).

An international unit is the quantity of IL-6 producing 50% of maximum cell growth.

In this paper, the measure is expressed as per cent recovery of the activity of sample IL-6 in the various formulations, on the 20 assumption that the sample activity at zero time is 100%.

Assays were carried out in duplicate.

Tables 2 and 3 show the results of assays conducted on the samples of Table 1 after 4, 5, 7, 8 and 9 weeks (Table 2) and after 10, 12, and 21 weeks (Table 3).

25 Samples A1 to A6 were subjected to immunologic assay (Table 2) and

samples A7 to A10 were subjected to bioassay (Table 3).

Table 2 - Stability at 50°C of IL-6 formulations A1 to A6 (35 µg) by immunologic assay, expressed as % recovery vs. zero time

Formulation	50°C				
	4W	5W	7W	8W	9W
A1			62	80	84
A2			78	80	80
A3			103	120	112
A4	81	82			
A5	107	104			
A6	89	100			

W = weeks

Table 3 - Stability at 50°C of IL-6 formulations A7 to A10 (35 µg) by bioassay, expressed as % recovery vs. zero time

Formulation	50°C		
	10W	12W	21W
A7	38	35	37
A8	104	95	74
A9	51	49	
A10	56	61	

W = weeks

The data shown in the Tables reported above demonstrate that the compositions containing nonreducing sugar, such as e.g. sucrose

or trehalose, (A3, A5, A6, A8) are much more stable than the compositions containing mannitol or lactose (A1, A2, A4, A6, A7). With a view to evaluating the effect of arginine, glycine or albumin on the formulations stability, IL-6 compositions containing sucrose or lactose alone vs. compositions containing the additional excipient were prepared (Table 4).

For the purpose of evaluating the effect of pH on the stabilizing action of the various components, the formulations were prepared by freeze-drying aqueous solutions at various pH (5.5, 6, and 7).

Table 4

Recombinant IL-6 formulations (35 µg) containing sucrose or lactose with or without additional excipient (content/vial)

Comp.	Saccharose	Lactose	Arginine	HCl	HSA	Na ₂ HPO ₄	NaH ₂ PO ₄	pH
Form.	mg	mg	mg	mg	mg	mg	mg	
B1	45					0.035	1.17	5.5
B2		45				0.035	1.17	5.5
B3	40.4		1.5			0.035	1.17	5.5
B4		40.4	1.5			0.035	1.17	5.5
B5	45			0.25	0.035	1.17		5.5
B6	45					0.107	1.11	6.0
B7		45				0.107	1.11	6.0
B8	40		1.5			0.107	1.11	6.0
B9		40	1.5			0.107	1.11	6.0
B10	45			0.25	0.107	1.11		6.0
B11	48					0.313	0.336	7.0
B12		48				0.313	0.336	7.0
B13	43.3		1.5			0.313	0.336	7.0
B14		43.3	1.5			0.313	0.336	7.0
B15	48			0.25	0.313	0.336		7.0

The stability of the above formulations was studied on samples maintained at 25°C and 50°C; the residual activity was measured at the time intervals shown in Tables 5 and 6. Table 5 illustrates the stability data of samples subjected to immunologic assay and Table

6 shows the stability data of samples subjected to bioassay.

Activity data are expressed as % recovery vs. zero time.

Table 5 - Comparison among stability data of IL-6 formulations (35 µg) containing sucrose or lactose with or without an additional excipient. (% recovery vs. zero time) - immunologic assay

Form.	25°C								50°C							
	2W	3W	4W	6W	7W	8W	9W	10W	2W	3W	4W	6W	7W	8W	10W	
B1	98		112						112		103					
B2			92						99		89					
B3	86		96						129							
B4	90								91		72					
B5	91		86						89		83					
B6		112								104		101				
B7		89		74						71						
B8		115		93						112		107				
B9		97		90						97		110				
B10	88		85						95							
B11		91	116	98	119					95		120		101		
B12		105				95				103						87
B13		107		102	100					87				80		
B14		103		86	92					94		119				
B15	107		11	103					105							

W = weeks

Table 6 - Comparison among stability data of IL-6 formulations (35 µg) containing sucrose or lactose with or without an additional excipient. (% recovery vs. zero time) - bioassay

	25 °C							50 °C								
	Form.	2W	3W	4W	5W	6W	7W	8W	9W	2W	3W	4W	5W	6W	7W	8W
B1	107				96	100				93	105				91	
B2	109				94	86				99	85				75	
B3	117				98					105	93	103				
B4	90					96				104	94				77	
B5	94	103				81				100	98				93	
B6	92		96	108						96	106			95	103	
B7	110			84						104		83	70			
B8	113		109	103			120			118	119		106		118	
B9	93		72							80	75	68				
B10	106				109					103	92			112		
B11	106	98				111				115		113		104		
B12	110	81				102				81		74		70		
B13	94			97						88			95			
B14	94			103						95		70	73	69		
B15	97				110						89			102		

W = weeks

As may be seen, the further excipients, i.e. arginine and albumin, added to the formulations containing sucrose and lactose participate in the stabilizing action to a negligible extent.

The data listed in Tables 5 and 6 also demonstrate that the formulations containing a nonreducing sugar, e.g. sucrose, show a much lower denaturation than those containing a reducing sugar, such as lactose.

5 Formulations at pH 7 and those at pH 5.5 or 6 show an analogous denaturation: it follows that, in the range considered, the influence of pH value on the formulation stability seems negligible. In any case, pH values approaching or equalling neutrality are preferred for the formulations for injection.

10 The formulation selected for an in-depth study contains sucrose, at pH 7. For the purpose of evaluating dosage influence on stability, two compositions containing different quantities of active ingredients were prepared (Table 7).

Table 7 - Formulations of recombinant IL-6 with sucrose (content/vial)

Comp. Form.	Saccharose mg	Na ₂ HPO ₄ /NaH ₂ PO ₄ mg	pH	IL-6 mg	
C1	48	0.313	0.336	7	0.035
C2	48	0.313	0.336	7	0.350

15 The investigation was carried out on samples stored in vials for 2, 4, 8, and 10 weeks at 25°C, 37°C, and 50°C. Stability was measured by immunologic assay expressed as per cent recovery of the sample activity at zero time (Table 8).

Table 9 recapitulates the stability of samples stored in vials for

4, 10, and 12 weeks at the aforesaid temperatures. Stability was measured by bioassay, still expressed as per cent activity recovery at zero time.

Table 8 - Study of the stability of IL-6 plus sucrose formulations. Per cent recovery vs. zero time - Immunologic assay

Form.	25°C				37°C				50°C			
	2W	4W	8W	10W	2W	4W	8W	10W	2W	4W	8W	10W
C1	105	90	94	97	111		94		101	95		90
C2		94	102			97	105			105	98	

W = weeks

Table 9 - Study of the stability of IL-6 plus sucrose formulations. Per cent recovery vs. zero time - Bioassay

Form.	25°C			37°C			50°C		
	4W	10W	12W	4W	10W	12W	4W	10W	12W
C1	91	100	92	80	90		92	96	94
C2	113			107			95		

W = weeks

As shown from the data of Tables 8 and 9, the denaturation of the formulations containing sucrose is extremely low and different IL-6 dosages do not affect the formulation stability.

The very low denaturation of the aforesaid compositions was confirmed by chromatographic analyses conducted on samples at the same time intervals and at the same temperatures as mentioned above.

Chromatographic analysis by molecular size separation was carried out with VARIAN MICROPAK TSK GEL G-3000 SW column (diameter: 7.5 mm, length: 30 cm) at a flow rate of 0.4 ml/min. The mobile phase was a 100 mM phosphate buffer at pH 6.85 and 11.69 g/l NaCl.

5 The analyses did not show any variation of the samples chromatographic profile in respect of zero time and confirmed that sucrose was the most appropriate excipient for IL-6 formulations stabilization.

EXAMPLES OF PHARMACEUTICAL PRODUCTS

10 Materials: extra pure sucrose Ph Eur, BP, Ph Nord, NF (Merck); reagent grade $\text{Na}_2\text{HPO}_4 \cdot 2\text{H}_2\text{O}$ (Merck), $\text{NaH}_2\text{PO}_4 \cdot \text{H}_2\text{O}$ (Merck); 0.1 M phosphoric acid (Merck); 0.1 M NaOH (Merck); water for injection. The containers used were DIN 2R glass vials (borosilicate glass, type I) sealed with Pharmagummi butyl rubber and aluminium ring.

15 Preparation of IL-6 solution containing sucrose (for 1000 vials containing 35 µg IL-6/vial)

Saccharose (48 g), $\text{Na}_2\text{HPO}_4 \cdot 2\text{H}_2\text{O}$ (0.313 g) and $\text{NaH}_2\text{PO}_4 \cdot \text{H}_2\text{O}$ (0.336 g) were dissolved in water for injection (400 ml) to form the initial sucrose solution. The obtained solution was divided into two equal parts. Recombinant IL-6 bulk (35 mg) was diluted with one solution part and adjusted to pH 7 with 0.1 M NaOH or H_3PO_4 . The two solutions were diluted to the final volume of 250 ml with water for injection.

20 The solution containing IL-6 was filtered through a 0.22 µ Durapore sterile filter and diluted to the final volume with the remaining

excipients solution, filtered through the same Durapore filter.

During the process, the solution temperature was maintained at 4°C to 8°C.

IL-6 solutions containing other excipients or a different quantity
5 of active ingredient were prepared following an analogous procedure.

Filling and freeze-drying

Vials were filled with 0.5 ml IL-6 solution, placed in the freeze-drier, and cooled to -45°C for 3 to 6 hours. Freeze-drying started at -45°C under 0.07 millibar vacuum. Heating scheme was as follows: +10°C for 10 to 12 hrs, then +30°C until the cycle end.

10 The reconstituted solution was subjected to the usual quality controls.

Although the present invention has been illustrated by specific examples, it is understood that variations to the applications described herein can be introduced without falling outside the
15 spirit and object thereof.

CLAIMS

1. Pharmaceutical composition containing an intimate solid mixture of Interleukin-6 (IL-6) and a stabilizing quantity of a nonreducing sugar either alone or in conjunction with other excipients.
1. 2. The pharmaceutical composition according to claim 1 wherein said intimate solid mixture is a freeze-dried powder.
1. 2. 3. The pharmaceutical composition according to claims 1 and 2 wherein said nonreducing sugar is sucrose or trehalose.
1. 2. 4. The pharmaceutical composition according to any of claims 1 to 3 wherein said IL-6 is recombinant.
1. 2. 5. The pharmaceutical composition according to any of claims 1 to 4 wherein said stabilizing agent is sucrose or trehalose alone.
1. 2. 6. The pharmaceutical composition according to any of claims 1 to 4 wherein said stabilizing agent is sucrose or trehalose in conjunction with an amino acid.
1. 2. 7. The pharmaceutical composition according to claim 6 wherein said amino acid is arginine.
1. 2. 8. The pharmaceutical composition according to any of claims 1 to 4 wherein said stabilizing agent is sucrose or trehalose in conjunction with albumin.
1. 2. 9. The pharmaceutical composition according to any of claims 1 to 8, containing 35 or 350 µg of IL-6 and 48 mg of sucrose.
1. 2. 10. The pharmaceutical composition according to any of claims 1 to 8, containing 35 or 350 µg of IL-6 and 47.5 mg of trehalose.
1. 2. 11. Procedure for the preparation of the pharmaceutical composition according to any of claims 1 to 10 comprising the following steps:

3 preparation of a components aqueous solution, distribution of same
4 into containers, and drying or freeze-drying of same in the
5 containers.

1 12. Procedure for the preparation of the pharmaceutical composition
2 according to any of claims 1 to 10, comprising the following steps:
3 preparation of a components aqueous solution, drying or freeze-
4 drying of same, and distribution of the solid mixture obtained into
5 containers.

1 13. The procedure according to claims 11 and 12 wherein the solution
2 pH ranges between 4.5 and 8.5.

1 14. The procedure according to claim 13 wherein the solution pH is
2 7.

1 15. The forms of pharmaceutical composition containing the intimate
2 solid mixture according to any of claims 1 to 10, hermetically
3 enclosed in a sterile container suitable for storage before use and
4 for the mixture reconstitution in a solvent or a solution for
5 injection.

1 16. Solution containing the solid mixture according to claim 15,
2 reconstituted in a solvent or a solution for injection.

INTERNATIONAL SEARCH REPORT

PCT/EP 93/01120

International Application No.

I. CLASSIFICATION OF SUBJECT MATTER (If several classification symbols apply, indicate all)⁶

According to International Patent Classification (IPC) or to both National Classification and IPC

Int.C1. 5 A61K37/02; A61K47/26; A61K47/42; A61K47/18
A61K9/14

II. FIELDS SEARCHED

Minimum Documentation Searched⁷

Classification System	Classification Symbols
Int.C1. 5	A61K ; C07K

Documentation Searched other than Minimum Documentation
to the Extent that such Documents are Included in the Fields Searched⁸III. DOCUMENTS CONSIDERED TO BE RELEVANT⁹

Category ¹⁰	Citation of Document, ¹¹ with indication, where appropriate, of the relevant passages ¹²	Relevant to Claim No. ¹³
Y	BIOPHARM vol. 3, no. 9, October 1990, pages 26 - 30 M. J. PIKAL 'Freeze-drying of proteins' cited in the application see the whole document especially page 28 left column ---	1-16
Y	EP,A,0 220 574 (YEDA RESEARCH AND DEVELOPMENT COMPANY, LIMITED) 6 May 1987 cited in the application see the whole document ---	1-16 -/-

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IV. CERTIFICATION

Date of the Actual Completion of the International Search

22 SEPTEMBER 1993

Date of Mailing of this International Search Report

01-10-1993

International Searching Authority

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Signature of Authorized Officer

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III. DOCUMENTS CONSIDERED TO BE RELEVANT (CONTINUED FROM THE SECOND SHEET)		
Category ^a	Citation of Document, with indication, where appropriate, of the relevant passages	Relevant to Claim No.
Y	WO,A,9 000 397 (CETUS CORPORATION) 25 January 1990 cited in the application see page 7, line 1 - line 12 see table I see claims ---	1-16
X	WO,A,9 116 038 (TORAY INDUSTRIES, INC.) 31 October 1991 see page 6, line 10 see claims ---	1
Y	Section Ch, Week 9111, 5 February 1991 Derwent Publications Ltd., London, GB; Class B04, AN 91-078688 & JP,A,3 027 320 (AJINOMOTO KK) 5 February 1991 see abstract ---	1-16
A	EP,A,0 378 171 (AJINOMOTO CO., INC.) 18 July 1990 see page 4, line 28 - line 30 ---	1-16
P,X	WO,A,9 306 840 (TORAY INDUSTRIES , INC.) 15 April 1993 see page 10, line 16 - page 11, line 11 -----	1-16

**ANNEX TO THE INTERNATIONAL SEARCH REPORT
ON INTERNATIONAL PATENT APPLICATION NO.**

EP 9301120
SA 75229

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Patent document cited in search report	Publication date	Patent family member(s)		Publication date
EP-A-0220574	06-05-87	AU-B-	602872	01-11-90
		AU-A-	6386786	16-04-87
		DE-A-	3688671	12-08-93
		EP-A-	0536520	14-04-93
		JP-A-	62263200	16-11-87
-----	-----	-----	-----	-----
WO-A-9000397	25-01-90	US-A-	5078997	07-01-92
-----	-----	-----	-----	-----
WO-A-9116038	31-10-91	JP-A-	5000963	08-01-93
		EP-A-	0477386	01-04-92
-----	-----	-----	-----	-----
EP-A-0378171	18-07-90	JP-A-	3101624	26-04-91
		US-A-	5126325	30-06-92
-----	-----	-----	-----	-----
WO-A-9306840	15-04-93	EP-A-	0560998	22-09-93
-----	-----	-----	-----	-----